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Full Length Research Paper

Antimicrobial activity and chemical composition of essential oils of *Stachys lavandulifolia* Vahl. from Mazandaran , Iran

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***Stachys lavandulifolia* vahl. or Chaye Koohi is a native plant, which has been used as an anxiolytic and sedative in Iranian folk medicine which belongs to the Lamiaceae family. In this study, *S. lavandulifolia* vahl. were collected from mountain of Baladeh area in Mazandaran province, Iran. Chemical constituents of essential oil of *S. lavandulifolia* vahl. were determined. Aerial parts were subjected to hydrodistillation (HD) in a Clevenger – type apparatus until there was no significant increase in the volume of the oil collected (3 h). The yield of the oil was 0.69% (w/w). The essential oil was analyzed by gas chromatography (GC) and GC-mass spectrometry (GC-MS). Identification of the components was based on GC retention indices computer matching with Wiley GC-MS library, and by comparison of the fragmentation patterns of the mass spectra with those reported in the literature. 59 components were identified constituting more than 99.1% of the oil. Hexadecanoic acid (13.9%), α-pinene (13.7%), germacrene-D (8.9%), β-pinene (7.0%), myrcene (4.5%), β-phellandrene (5.7%), *Z*-ocimene (3.4%), spathulenol (3.5%), δ-cadinene (2.0%) and α-cadinol (2.6%) were major components in *S. lavandulifolia* vahl. oil. The oil was tested against two strains of bacteria (Gram-positive and Gram-negative). *In vitro* antimicrobial activity of essential oil of *S. lavandulifolia* vahl. were investigated by disc diffusion method and the minimum inhibitory concentration (MIC) and also minimal bactericidal concentration (MBC) determination. The studied sample was active against gram-positive and Gram-negative microbial strains. The oil exhibited higher activities against the Gram-negative tested bacterial strain.**

Key words: *Stachys lavandulifolia* vahl., essential oil composition, antimicrobial activity, α-pinene.

INTRODUCTION

The genus *Stachys* (Lamiaceae) is represented by about 300 species found in the world, mostly in Europe and Asia (Evans, 1996). It has been represented in Iran by 34 species including 13 endemics (Mozaffarian, 2007). The *Stachys* species belong to one of the oldest medicinal plants that are used both for pharmaceutical purposes and in folk medicine; and also the plants of this genus have long been applied to treat genital tumors, sclerosis of the spleen, inflammatory tumors and cancerous ulcers (Skaltsa et al., 1999). This genus contains different natural product classes, including monoterpenes, sesquiterpenes, diterpenes, triterpene saponins, flavonoids, biflavonoids, glycosides, and phenolic acids

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(Chalchat et al., 2001; Kobzar, 1986; Kotsos et al., 2001; Miyase et al., 1996; Paternostro et al., 2000; Yamamoto et al., 1994). *Stachys lavandulifolia* Vahl. is a native plant and used as herbal tea; it has been known as Chaye Koohi and also Sonbole Ziba and widely distributed in Iran, which also has been used as an anxiolytic and sedative in Iranian folk medicine (Amin, 1991). It has been reported to contain volatile oil and a phenyl propanoid glycoside (Basaran et al., 1988; Sezik and Basaran, 1985).

Many studies of the essential oil and extracts content of *Stachys* species have been performed. The essential oils of the dried flowering aerial parts of *Stachys byzantina, Stachys inflata, Stachys lavandulifolia* and *Stachys laxa* collected from Iran were isolated in *S. lavandulifolia* oil, the major compounds were 4-hydroxy-4-methyl-2-pentanone, α-pinene and hexadecanoic acid (Morteza-Semnani et al., 2006). The antimicrobial activity of the methanol extracts of the dried flowering aerial parts of *S. byzantina, S. inflata, S. lavandulifolia* and *S. laxa* were studied using the disc diffusion method and minimum inhibitory concentration (MIC) values against a panel of microbial strains and several fungal species (Saeedi et al., 2008). The extracts of plants exhibited concentration-dependent antibacterial activity against the bacteria tested. The extracts were more active against gram-positive microorganisms. The extracts, however, did not show any antifungal activity.

The effects of extract and essential oil of *S. lavandulifolia* Vahl. on the elevated plus-maze (EPM) model of anxiety has been investigated (Rabbani et al., 2003). The *S. lavandulifolia* extract or its essential oil, at various doses, was administered intraperitoneally to male mice, and demonstrated that the extract produced hypnotic and sedative activities.

The elucidate therapeutic and preventive effects of *S. lavandulifolia* extract collected from Kordestan province, Iran, on gastric acid and pepsin secretions in experimental gastric ulcer has been investigated (Nabavizadeh et al., 2011). Thirty-two (32) Wistar male rats were used to study therapeutic and preventive effects of *S. lavandulifolia* extract on alcohol-induced gastric ulcer. The results showed that *S. lavandulifolia* extract protected gastric mucosa from alcohol-induced gastric ulcer. Monji et al. (2011) evaluated the toxic effects of *S. lavandulifolia* hydroalcoholic extract in female mice in acute and subchronic models. To assess the toxicity profile, this extract was administered by oral gavages in acute, subacute and subchronic models. By assessing all clinical, hematological, biochemical and histopathological changes, maximum tolerate were recognized in subchronic model.

Composition and antioxidant and antimicrobial activities of essential oil and methanol extract of *S. inflata* have been determined (Ebrahimabadi et al., 2010). It showed 45 constituents representing 95.46% of the oil, the major components were linalool (28.55%), α-terpineol (9.45%), spathulenol (8.37%) and (2E)-hexenal (4.62%). The plant also showed a week antimicrobial activity against three strains of tested microorganisms. Linalool and α-terpineol were also tested as major components of the oil and showed no antioxidant but considerable antimicrobial activities.

The ethanol extract of the leaves of *Stachys pseudopinardii* R. Bhattacharjee and Hub.–Mor. from turkey were investigated for their antimicrobial activities (Dulger and Aki, 2009). The extracts showed strong antibacterial activity against *Bacillus cereus*, and MIC and minimum bactericidal concentration (MBC). The extract exhibited moderate activity against the other test microorganisms. The results demonstrate that the ethanol extract of the leaves of *S. pseudopinardii* has significant antimicrobial activity and suggest that it may be useful in the treatment of infections. The antimicrobial activities of methanolic extracts of some Lamiaceae members (such as *Stachys woronowii* R. Mill.) collected from Turkey have been reported (Kursat and Erecevit, 2009). Results indicated that plant extracts inhibited the growth of tested microorganisms in the different ratio; however, some plant extracts had no effect against tested microorganisms.

Therefore, the aim of this study was to evaluate the essential oil composition and antimicrobial activity of *S. lavandulifolia* Vahl. in flowering stage which grows wild in Mazandaran province, Iran (Figure 1).

MATERIALS AND METHODS

Plant material

S. lavandulifolia Vahl. (Figure 1) in flowering stage were collected from Baladeh Mountain area (Mazandaran province, Iran) at an altitude of ca. 2400 m (latitude +36ǂ12'14"N, longitude +51ǂ47'58"E), in June, 2010. Voucher specimens of the plant have been deposited in the herbarium. The plants were air dried and dried samples were crushed; then essential oils were obtained by hydrodistillation (HD).

Isolation of the essential oil

Air-dried aerial parts plant material (100 g) was submitted to water distillation for 3 h using an all-glass Clevenger-type apparatus as recommended by the European Pharmacopoeia (Anonymous, 1996) and yielded essential oil 0.69% (w/w) of dry matters. After decanting, the obtained essential oils were dried over anhydrous sodium sulfate and after filtration, stored in refrigerator at - 4°C until tested and analyzed.

Analysis of the essential oil

Gas chromatography

The oil was diluted in acetone (1:9) and 1 µl was used for analysis. Gas chromatography-mass spectrometry (GC-MS) analyses of the essential oil was analyzed on an Agilent Technologies 7890A GC system coupled to a 5975C VLMSD MS with an injector 7683B series device. An Agilent (9091) 413:325°C HP-5 column (30 m ×

Figure 1. Photo of *S. lavandulifolia* Vahl. from Baladeh aera in Mazandaran, Iran.

320 µm × 0.25 µm) was used with helium as carrier gas at a flow rate of 3.35 ml/min. The GC oven temperature was initially programmed at 50°C (hold for 1 min) and finally at 300°C (hold for 5 min) at a rate of 80°C/min while the trial temperature was 37.25°C.

The column heater was set at 250°C in a split less mode while the pressure was 10.2 psi with an average velocity of 66.5 cm/s and a hold-up time of 0.75 min. MS was run in the electron impact mode (EI) at 70 eV. The percentage compositions were obtained from electronic integration measurements using flame ionization detector (FID), set at 250°C.

Gas chromatography-mass spectrometry

The essential oil was analyzed by GC-MS on an Agilent Technologies 7890A GC system coupled to a 5975C VLMSD mass spectrometer with an injector 7683B series device. An Agilent (9091) 413:325°C HP-5 column (30 m × 320 µm × 0.25 µm) was used with helium as carrier gas at a flow rate of 3.35 ml/min. GC oven temperature and conditions were the same as previously described. The injector temperature was at 250°C. Mass spectra were recorded at 70 eV. Mass range was from 30 to 500 m/z.

Identification of components

Identification of components was based on their retention indices determined by reference to a homologous series of n-alkenes, and by comparison of their mass spectral fragmentation patterns with those reported in the literature (Adams, 2007), and stored on the MS library (NIST 08.L database/ chemstation data system) with data previously reported in literature (McLafferty and Stauffer, 1989; Joulain and Konig, 1998; Jennings and Shibamoto, 1980). The percentages of each component are reported as raw percentages based on total ion current without standardization. The chemical compositions of essential oil of *S. lavandulifolia* Vahl. are shown in Table 1 and Figures 2 and 3.

Antimicrobial assay of the oil

In vitro antibacterial assay of the oil was carried out according to disc agar diffusion method (Jirovets, 1999; Kumar et al, 2004). Antibacterial activity of the oil was tested against gram-positive bacterial microbial strains *Staphylococcus aureus* (PTCC1431) and gram-negative bacteria strain *Escherichia coli* (PTCC1399), were provided by Iranian Research Organization for Science and Technolog (IROST), and grown in nutrient broth for 24 h (pH 7.2 to 7.4), and were used as inoculums. Mueller Hinton agar was used as the bacteriological medium. The Mueller-Hinton agar medium were poured into the plates to uniform depth of mm and allowed to solidify. The oils and extracts were diluted in 5% dimethyl sulfoxide (DMSO). Then the microbial suspensions were streaked over the surface of media using a sterile cotton swab to ensure the confluent growth of the organism. Aliquots of 10 µl of the oil at 1:2 dilutions in DMSO were impregnated on Whatman No. 1 filter paper discs of 6 mm diameter. These discs were aseptically applied to the surface of the agar plates at well-spaced intervals. The plates were incubated at 37°C for 24 h and observed inhibition zones including the diameter of the discs were measured. Five controls discs were also included in the test; the first was involving the presence of microorganisms without test material and other were standard antibiotics: erythromycin, penicillin, streptomycin, and chloramphenicol were used to control the sensitivity of the tested microorganisms. Control discs impregnated with 10 µl of the solvent DMSO and antibiotics (10 to 30 µg/disc), reference for bacteria were used alongside the test discs in each experiment. The experiments were performed in triplicates. The results are shown in Table 2 and Figure 4.

MIC) and MBC were determined for the oil and showed total growth inhibition using the method previously described. Oil concentrations of 0.1 to 25.0 mg/ml were evaluated. The MIC value was defined as the lowest concentration producing no visible growth (absence of turbidity and or precipitation) as observed through the naked eye and the concentration at which there was no bacterial growth after inoculation in Müeller-Hinton agar was taken as the MBC (AL.Janabi, 2011) . The experiments were performed in triplicates and repeated twice. Erythromycin, penicillin, streptomycin, and chloramphenicol were used as positive controls whilst 5% DMSO-broth mixture was used as the negative control. All the results were recorded as the mean concentration of triplicate(Table 3 and Figure 5). The standards were selected based on their availability and their presence in the oil studied.

Table 1. Chemical composition (%[a]) of the essential oils isolated from the aerial parts of *S. lavandulifolia* Vahl.

Compound[b]	Aerial parts	KI[c]	Method of identification[c]
α-Thujene	1.0	907	MS, KI
α-Pinene	13.7	924	MS, KI
Camphene	0.3	954	MS, KI
β-Pinene	7.0	979	MS, KI
Myrcene	4.5	990	MS, KI
α-Phellandrene	0.4	1002	MS, KI
δ- 3-carene	0.1	1011	MS, KI
α-Terpinene	0.2	1017	MS, KI
β-Phellandrene	5.7	1029	MS, KI
1,8-Cineole	0.4	1031	MS, KI
Z-α-Ocimene	3.4	1037	MS, KI
E-β-Ocimene	0.4	1050	MS, KI
γ-Terpinene	0.9	1059	MS, KI
Z-sabinene hydrate	0.1	1070	MS, KI
Terpinolene	0.2	1088	MS, KI
Linalool	0.2	1096	MS, KI
Terpinene-4-ol	0.2	1177	MS, KI
α-Terpineol	0.2	1188	MS, KI
Bornyl acetate	0.1	1288	MS, KI
α-Cubebene	2.2	1348	MS, KI
Geranyl acetate	1.9	1381	MS, KI
β-Bourbonene	0.8	1387	MS, KI
β-Cubebene	0.3	1388	MS, KI
β-Elemene	0.8	1390	MS, KI
Caryophyllene	1.3	1420	MS, KI
E-β-Farnesene	0.5	1456	MS, KI
α-Amorphene	0.2	1483	MS, KI
Germacrene D	8.9	1485	MS, KI
Valencene	0.3	1496	MS, KI
Bicyclogermacrene	2.2	1500	MS, KI
β-Bisabolene	0.7	1505	MS, KI
Z-α-Bisabolene	0.7	1507	MS, KI
γ-Cadinene	0.5	1513	MS, KI
Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene	0.4	1518	MS, KI
δ-Cadinene	2.0	1523	MS, KI
Spathulenol	3.5	1578	MS, KI
Veridiflorol	0.5	1592	MS, KI
Salvial-4(14)-en-1-one	0.5	1594	MS, KI
1-4 Methano-1H-indene, octahydro-1,7a-dimethyl-4-(1-methylethenyl	0.7	1609	MS, KI
Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)	0.1	1621	MS, KI
1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene	0.3	1630	MS, KI
α-Cadinol	2.6	1654	MS, KI
1,1,4,4-Tetramethyl-2-tetralone	1.2	1679	MS, KI
Anymol	0.6	1690	MS, KI
Iso-Caryophyllene	2.2	1699	MS, KI
4-Bromo-1-naphthalenamine	0.1	1723	MS, KI
Tetradecanoic acid	0.6	1775	MS, KI
2-Pentadecanone, 6,10,14-trimethyl	1.4	1845	MS, KI
E-β-Santalol acetate	0.3	1865	MS, KI

Table 1. Contd.

Phytol	1.2	1943	MS, KI
Hexadecanoic acid	13.9	1960	MS, KI
Eicosane	0.1	2000	MS, KI
n-Henicosane	0.2	2100	MS, KI
Linoleic acid	2.5	2133	MS, KI
Pentacosane	0.4	2500	MS, KI
Compound 889	0.2	2542	MS, KI
Heptacosane	1.3	2700	MS, KI
Octacosane	1.3	2800	MS, KI
Triacontane	0.7	3000	MS, KI
Number of identified compounds		59	
Yield of the oil (w/w %)		0.69	
Monoterpene hydrocarbons		37.5	
Oxygenated monoterpenes		3.1	
Sesquiterpene hydrocarbons		21.4	
Oxygenated sesquiterpenes		10.2	
Oxygenated diterpenes		1.4	
Others components		25.2	
Total identified		99.1	

[a]%, Peak area of essential oil components; [b] KI, Kovats indices on HP-5 capillary column in reference to C_8-C_{28} n-alkanes (Adams, 2007); [c], components were identified on KI and GC-MS (gas chromatograph coupled with mass spectrometry) and listed according to their elution on HP-5 MS capillary column (30 m).

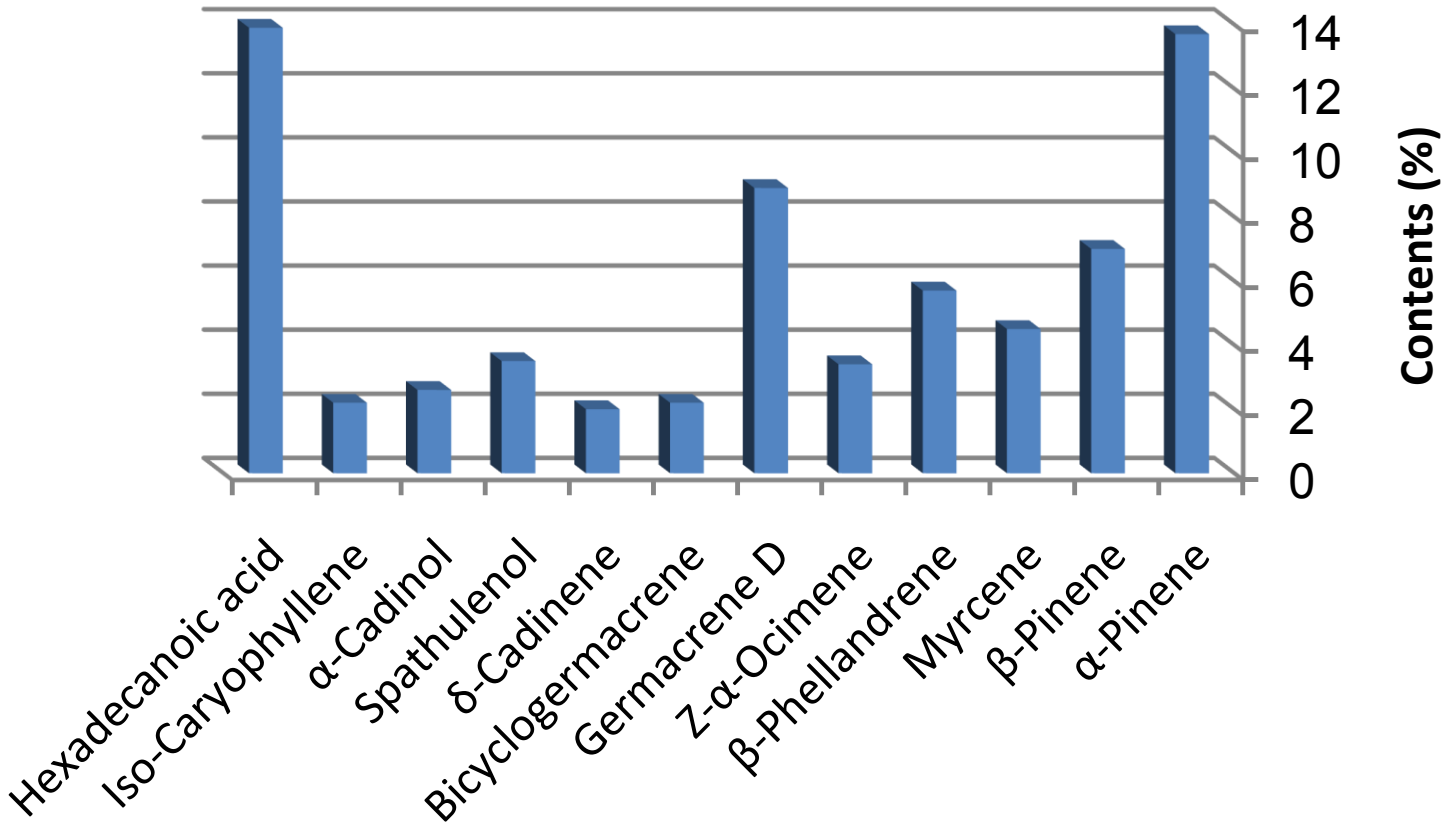

Figure 2. The major components of essential oil of *S. lavandulifolia*.

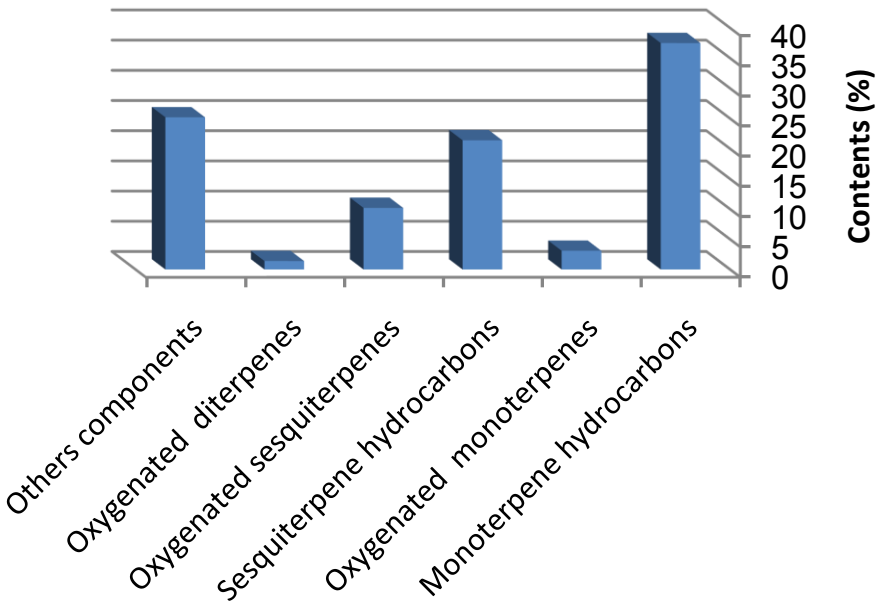

Figure 3. Percentage of main components in essential oil of *S. lavandulifolia.*

Table 2. Antibacterial activity (zoon inhibition diameter) of the oil of *S. lavandulifolia* Vahl. against two gram-positive and gram-negative bacteria as compare standard antibiotics.

			Zoon inhibition diameter (mm)				
Bacteria	**Gram**	**PTCC**	**Essential oil**	**Erythromycin (10 µg)**	**Penicillin (10 µg)**	**Streptomycin (15 µg)**	**Chloramphenicol (30 µg)**
S.aureus	Positive	1431	16	28	23	14	26
E. coli	Negative	1399	26	9	Not growth	17	25

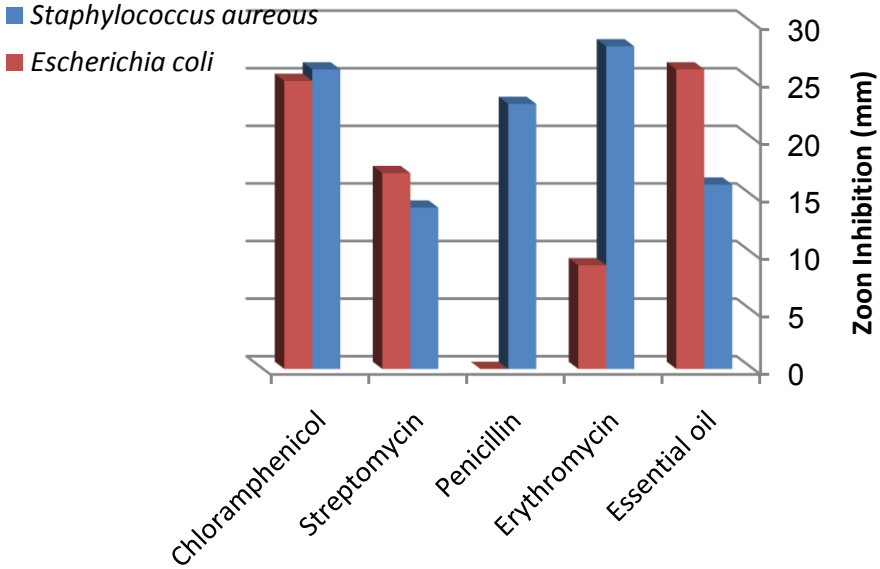

Figure 4. Zoon inhibition diameter of essential oil of *S. lavandulifolia* and standard antibiotics.

Table 3. Determination of MIC and MBC of *S. lavandulifolia* for bacteria.

Bacteria	Gram	PTCC	MIC (mg/ml)	MBC (mg/ml)
S. aureus	Positive	1431	4.3	8.0
E. coli	Negative	1399	2.15	4.3

MIC, Minimum inhibitory concentration; MBC, minimum bactericidal concentration.

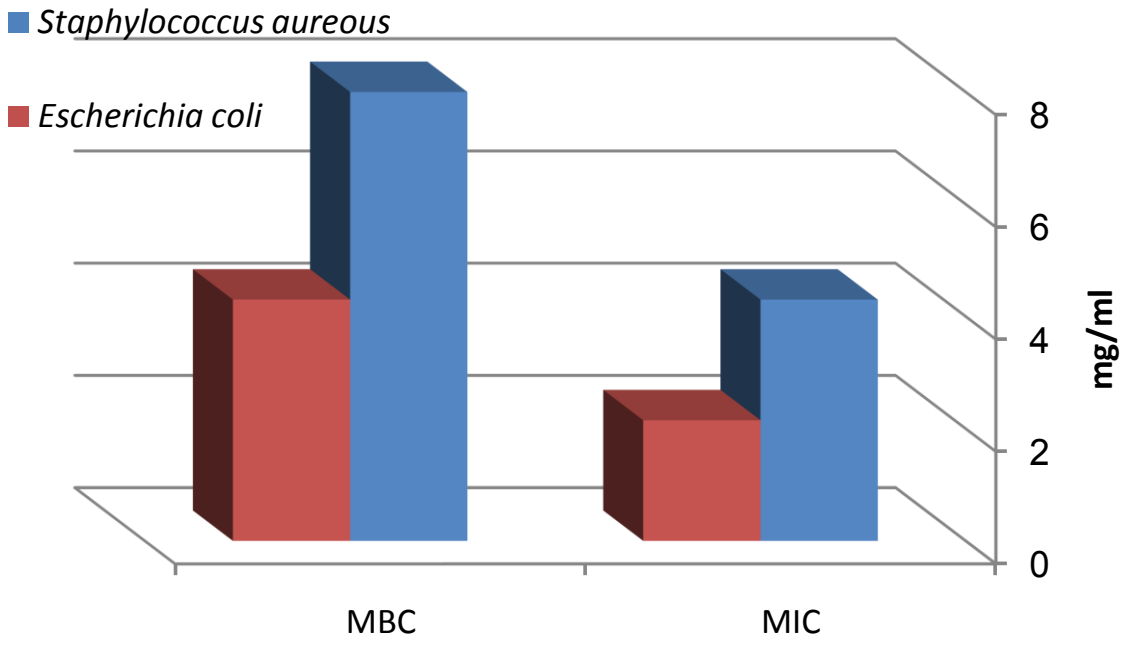

Figure 5. MIC and MBC values (mg/ml) of essential oil of *S. lavandulifolia*.

RESULTS

Essential oil composition

The essential oil was obtained from the 100 g crushed aerial parts of herbal plant (*S. lavandulifolia* Vahl.) by HD, which was immediately analyzed by both GC and GC-MS system. The volatile oil obtained in 0.69% (w/w) yield from air dried aerial parts of *S. lavandulifolia*. 59 components were identified constituting more than 99.1% of the oil. Among them, monoterpenes and sesquiterpenes were major terpenes (37.5 and 21.4%, respectively), whereas oxygenated monoterpene, oxygenated sesquiterpenes and oxygenated diterpenes were 3.1, 10.2 and 1.4%, respectively, and also other components were 25.2%.

Hexadecanoic acid (13.9%), α-pinene (13.7%), germacrene-D (8.9%), β-pinene (7.0%), myrcene (4.5%), β-phellandrene (5.7%), Z-ocimene (3.4%), spathulenol (3.5%), δ-cadinene (2.0%) and α-cadinol (2.6%) were major components in *S. lavandulifolia* Vahl. oil. The results are shown in Table 1 and Figures 2 and 3.

Antimicrobial activity

Inhibition zone diameter

In this study, effectiveness of essential oil was also confirmed by filter paper disc diffusion assay and growth inhibitions zone diameters were measured in presence and absence of essential oil. Results are shown in Table 2 and Figure 4.

The inhibitory effect was compared with standard antibiotics: erythromycin, penicillin, streptomycin, and chloramphenicol (10 to 30 µg/disc). The oil has shown larger growth inhibition zone diameters (26 mm) against gram-negative tested microorganism, *E. coli*, in comparison to gram-positive tested microorganism (16 mm), *S. aureus*, while inhibition zone diameters of standard antibiotics for erythromycin, streptomycin and chloramphenicol against *E. coli* were 9, 17 and 25 mm, respectively. The inhibition zone diameters of standard antibiotics against another microorganism, *S. aureus,* were: 28 mm for erythromycin, 23 mm for penicillin, 14mm for streptomycin and 26 mm for chloramphenicol.

Determination of MIC and MBC values

The results of antimicrobial activity (MIC and MBC) of the *S. lavandulifolia* Vahl. essential oil against two Gram-positive and Gram-negative bacterial strains are shown in Table 3 and Figure 5. The MIC and MBC determinations were obtained by disc agar diffusion method. In general, the oil showed moderate activity against two strains tested microorganisms. The results for essential oil indicated that MIC value for tested microorganisms was 2.15 mg/ml against *E. coli*, while MBC value was 4.3 mg/ml; on the other hand, MIC and MBC, against *S. aureus*, were 4.3 and 8.0 mg/ml, respectively.

DISCUSSION

In this study, hexadecanoic acid (13.9%), α-pinene (13.7%), germacrene-D (8.9%), β-pinene (7.0%), myrcene (4.5%), β-phellandrene (5.7%), *Z*-ocimene (3.4%), spathulenol (3.5%), δ-cadinene (2.0%) and α-cadinol (2.6%) were major components in *S. lavandulifolia* Vahl. oil, collected from Baladeh area in Iran. Amongst them, monoterpenes and sesquiterpenes were major terpenes, whereas oxygenated monoterpene, oxygenated sesquiterpenes and as well as oxygenated diterpenes were minor. The oil has shown larger growth inhibition zone diameters against gram-negative tested microorganism, *E. coli*, compared with gram-positive tested microorganism, *S. aureus*. The oil showed moderate activity against two tested microorganisms, MIC value for tested microorganisms against *E. coli* was less in comparison with *S. aureus*; moreover, MBC value against *S. aureus*, was more compared with *E. coli.*

The antibacterial activity of ethanol extracts and essential oils of 10 medicinal plants such as *S. lavandulifolia* against *Steptococcus iniae* was evaluated by disc diffusion assays. Most of the extracts and essential oils showed a relatively high antibacterial activity against *S. iniae* (Ghasemi et al., 2011).

In another study, extraction and composition determination of essential oil of *S. lavandulifolia* Vahl. from Qom province, Iran, by HD and microwave assisted hydrodistillation (MWHD) extraction methods were successfully performed. It has been shown that isolation; extraction and concentration of essential oil in fresh *S. lavandulifolia* can be done by two methods separately. Forty-seven (47) compounds were identified in the *S. lavandulifolia* by using the proposed methods. The experimental results demonstrated that using much less sample amount, shorter extraction time and simpler procedure, MWHD methods can achieve comparable results with those by HD for determination of essential oils in fresh materials. The major components by two methods were carvacrol and thymol (1.43, 2.63% and 10.80, 8.14%, respectively (Sadrmomtaz et al., 2011). In addition, germacrene-D, β-phellandrene, β-pinene, myrcene, α-pinene and Z-β-ocimene were also its other major components.

Chemical composition of the essential oil of *S. lavandulifolia* (after flowering) collected from Darkesh area (North Khorassan province, Iran) has been reported (Nadaf et al., 2011). The main components were bis (2-ethylhexyl), phthalate (58.39%), decane (25.46%), p-xylene (4.2%), dodecane (3.85%) and α-pinene (3.29%). These results are not in accordance with our finding.

The volatile compounds of 18 populations of the 6 taxa of *Stachys* subsect. Swinsonianeae were investigated by GC-MS analysis (Skatlas et al., 2001). All the investigated *Stachys* taxa essential oils ranged from 0.16 to 0.38% based on dry weight. All the essential oils are complex mixtures of more than 100 constituents. Among them sesquiterpenes consist the main portion in all studied taxa.

The composition of the essential oil obtained by HD from the leaves of *Stachys schtschegleevii* Sosn. were collected from Ahar (East Azerbaijan province, Iran) in June, 2003 before the flowering stage was analyzed by GC and GC-MS (Sonboli et al., 2005). Forty-five (45) compounds representing 98.7% of the total oil were identified, of which α-pinene (36.4%), germacrene-D (18.6%), limonene (8.2%) and piperitone (6.2%) were the major constituents. Furthermore, antibacterial activity of the entire oil and its two main monoterpenes was evaluated against 6 gram-positive and gram-negative bacteria. The oil exhibited moderate activity against the tested bacteria.

Anti-*Candida* activity of ethanolic extracts of Iranian endemic medicinal herbs against *Candida albicans* has been studied (Rohi-Boroujeni et al., 2012). Among them, one extract of native medicinal herb (*S. lavandulifolia* Vahl.) collected from Chaharmahal va Bakhtiari province in Iran, were assayed for the *in vitro* antifungal activity against *C. albicans*, using agar dilution methods. Most of the extracts showed relatively high anti-*Candida* activity against the tested fungi with the diameter of inhibition zone ranging between 8 and 17 mm. The MIC values for active extract range between 25 and 50 µg/ml.

The essential oils of the dried flowering aerial parts of *S. lavandulifolia* collected from the suburb of Behshahr, (north of Iran), in May, 2003, were isolated by HD and analyzed by means of GC and GC-MS. The major compounds of *S. lavandulifolia* oil were 4-hydroxy-4-methyl-2-pentanone (9.3%), α-pinene (7.9%) and hexadecanoic acid (5.2%) (Morteza-Semnani et al., 2006).

The constituents of the oil obtained by HD of *S. lavandulifolia* from central of Iran was analyzed using GC and GC/MS. Fifty-five (55) compounds has been observed, of which 44 could be identified. The major components has been found in the oil were α-pinene (20.1%), β-pinene (12.1%) and spathulenol (7.2%) (Feizbaksh et al., 2003).

The essential oil of the aerial parts of different stages of

growth as pre-flowering, flowering and post-flowering of *S. lavandulifolia* Vahl. from Lorestan province in Iran has been isolated by HD. The chemical composition of volatile oil was analyzed by capillary GC and GC-MS. The main components were found to be: α-pinene (27.25, 25.66 and 8.52%), myrcene (17.33, 9.33 and 23.85%), β-phellandrene (21.96, 37.49 and 12.58%) and β-caryophylene (14.3, 8.38 and 16.86%) (Sajjadi and Amiri, 2007). The essential oil of *S. lavandulifolia* Vahl. was collected in May, 2002 from the Fasham area near Tehranh, Iran, has been isolated by HD of the aerial parts of the plant, with a yield of 0.25%. The chemical composition of volatile oil was analyzed by capillary GC and GC-MS. The main components were germacrene-D (13.2%), β-phellandrene (12.7%), β-pinene (10.2%), myrcene (9.4%), α-pinene (8.4%) and Z-β-ocimene (5.8%) (Javidnia et al., 2004).

Conclusion

Previous reports of the oils of other *Stachys* species demonstrated varying compositions. For example, the oil of *S. lavandulifolia* Vahl. from central Iran has been studied and its main components were reported to be α-pinene (20.1%), β-pinene (12.1%) and spathulenol (7.2%) (Feizbakhsh et al., 2003), whereas in this study, *S. lavandulifolia* Vahl. from Mazandaran province in Iran, demonstrated hexadecanoic acid, α-pinene, germacrene-D, β-pinene, Myrcene, β- phellandrene, Z-ocimene, spathulenol, δ- cadinene and α-cadinol as major components which are different from some species that were reported (Nadaf et al., 2011; Sajjadi and Amiri, 2007; Morteza-Semnani et al., 2006). This study reveals the antimicrobial susceptibility of essential oil of *S. lavandulifolia* Vahl. against two bacteria. It is proved by low MIC and MBC values obtained in oil when used against each bacterial culture. MIC value for tested organisms against gram-negative bacteria (*E. coli*) was less against gram-positive bacteria (*S. aureus*); also MBC value against *S. aureus*, was more than *E. coli*.

The high concentration of α-pinene in *S. lavandulifolia* Vahl. revealed antimicrobial activity in this study and in the previous reports on other *Stachys* species (Grujic-Jovanovic et al., 2004). The oil at that concentration showed moderate activity against the two tested microorganism, but gram-negative microorganism, *E. coli*, was more sensitive to oil than other one. Whereas activity detected against the examined gram-positive microorganism, *S. aureus*, was found to be less sensitive to the oil. The finding also showed that the studied oils have relatively good and different antibacterial activity without significant toxicity, thus, have great potentiality to be used as natural health product and is considerable as an antibacterial agent in drug and food industries. Meanwhile, it can be said that the essential oils of some of the Iranian endemic medicinal plants could be used as natural anti-bacteria.

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